

Membrane sensory proteins in bacteria: how sensor kinases sense and respond to environmental signals in pathogenic and other bacteria.

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Introduction

In bacteria, sensing and responding to changing environmental conditions is mainly performed by two-component signal transduction systems (TCSs). These systems are crucial for survival in natural environments, or hosts that fluctuate with respect to a wide range of factors, and not surprisingly they are almost ubiquitous amongst bacteria. In pathogenic species, TCSs are pivotal in regulating virulence factor gene expression, and it has been proposed that TCSs constitute excellent new targets for the design of novel antibacterial drugs. TCSs comprise a sensor kinase (SK, or histidine protein kinase) and a response regulator (RR). The SK is usually located in the membrane and is responsible for stimulus perception and signal transduction (via phosphorylation) to the partner RR which then effects an appropriate response. Structural and functional information about membrane SKs is generally lacking, yet this is an important area for elucidating mechanisms of signal 'sensing' by bacteria, and signal transduction across the membrane. No structures of intact membrane SKs have yet emerged. Moreover, knowledge of the signals (environmental cues) themselves is also often lacking. This lack of information arises, in part, because of previous technical challenges in isolating and purifying membrane proteins, including SKs. In collaboration with Peter Henderson's group, we have succeeded in routinely purifying large quantities of membrane SK proteins for structural and functional studies. Progress in the past year is detailed below.

Redox-responsive *in vitro* modulation of the signalling state of the isolated PrrB sensor kinase of *Rhodobacter sphaeroides*.

The global redox switch between aerobic and anaerobic growth in *Rhodobacter sphaeroides* is controlled by the PrrA/PrrB two-component system (also known as the RegA/RegB), in which PrrB is the integral membrane sensor kinase, and PrrA is the cytosolic response regulator. We were the first group to successfully heterologously overexpress the intact membrane protein component, PrrB (or indeed any membrane sensor kinase), using technologies successfully developed by the Henderson group for other groups of membrane proteins.

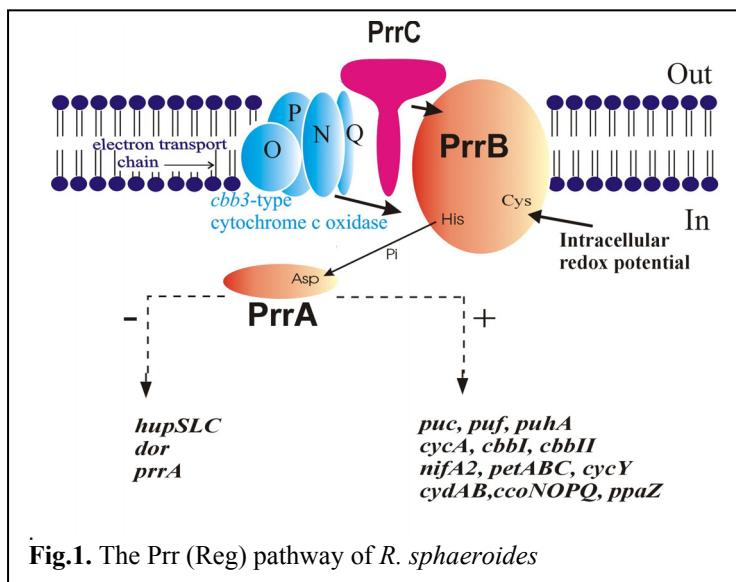


Fig.1. The Prr (Reg) pathway of *R. sphaeroides*

In the case of PrrB, the signal is redox potential, and this signal is generated from within the membrane itself (via the respiratory *cbb₃*-type cytochrome *c* oxidase and/or another membrane protein PrrC), and/or intracellularly. Previously, we showed that the overexpressed intact protein is functional both in *E. coli* inner membranes and as purified protein, as shown by its autophosphorylation, phosphotransfer and PrrA-dephosphorylation activities. Our kinetic data also revealed that the transmembrane region has important regulatory activity

Recently we showed that intact PrrB also retains its ability to sense and respond to its redox signal, paving the way for investigations of structural changes occurring during signal sensing. Fig 2 demonstrates that purified PrrB autophosphorylates in response to reducing conditions induced by reversible thiol exchanger dithiothreitol. This contrasts with a previous study of soluble PrrB (lacking its transmembrane region) which reported only weak increases in PrrB~P in response to DTT-induced reducing conditions. Fig 2 also shows that the higher levels of PrrB~P obtained in these experiments resulted in a concomitant increase in phosphotransfer to PrrA, resulting in higher levels of PrrA~P in our phosphotransfer assays. This therefore confirms that subsequent signal transduction from PrrB to PrrA occurs successfully. That the intact protein also phosphorylates as predicted in response to DTT-induced reducing conditions in the membrane environment is confirmed in Fig 3.

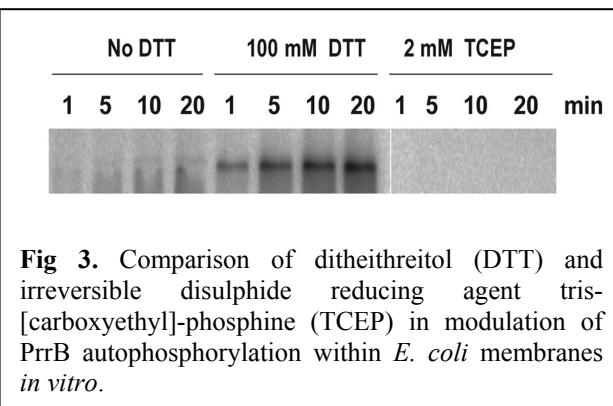


Fig 3. Comparison of ditreothreitol (DTT) and irreversible disulphide reducing agent tris[carboxyethyl]-phosphine (TCEP) in modulation of PrrB autophosphorylation within *E. coli* membranes *in vitro*.

complexes. The ability to produce milligram quantities of highly purified PrrB protein is also enabling us to undertake 2D/3D crystallisation in order to elucidate the 3D structure of this sensor kinase by electron or X-ray diffraction.

Overexpression and/or purification of virulence-associated intact SKs from pathogenic bacteria.

This year, we have successfully overexpressed and/or purified a further four, full-length membrane sensor kinases, all of which are known or candidate regulators of virulence factor or antibiotic resistance genes in pathogenic species. In addition to PrrB above, these membrane SKs will also be included in structural studies to elucidate their 3D structure.

Publications

Saidijam M., Psakis, G., Clough, J.L., Meuller, J., Suzuki, S., Hoyle, C.J., Palmer, S.L., Morrison, S.M., Pos, M.K., Essenberg, R.C., Maiden, M.C.J., Abu-bakr, A., Baumberg, S.G., Stark, M.J., Ward, A., O'Reilly, J., Rutherford, N.J., Phillips-Jones M.K. and. Henderson, P.J.F. (2003) Collection and characterisation of bacterial membrane proteins. *FEBS Lett.* **555**, 170-175.

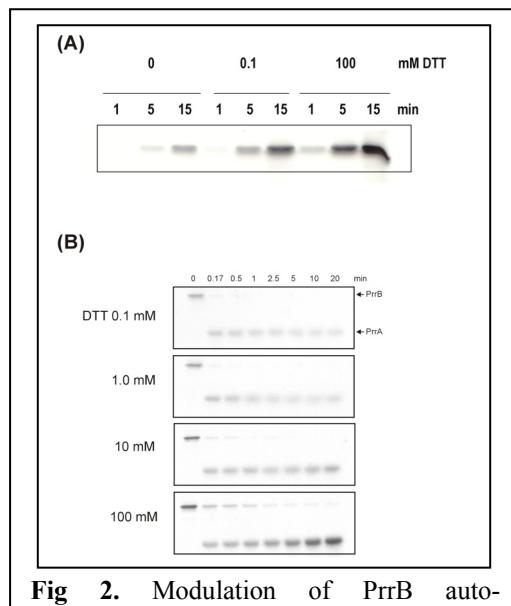


Fig 2. Modulation of PrrB autophosphorylation and phosphotransfer to PrrA by dithiothreitol *in vitro*.

In the past year, we have identified at least one redox-responsive, membrane-bound protein of *R. sphaeroides* that further modulates PrrB phosphorylation kinetics, and that may therefore regulate levels of PrrB~P *in vivo*. This data has recently been submitted for publication. The system is therefore giving insights into the signal sensing mechanisms. Targetted mutagenesis will facilitate elucidation of the structure-activity relationships of the single PrrB, PrrA and regulatory proteins as well as their

Jeong, E-L., Potter, C.A., Dupeux, E., Day, A.M., Williamson, M.P., Henderson, P.J.F. and Phillips-Jones, M.K. Redox-responsive *in vitro* modulation of the signalling state of the isolated PrrB sensor kinase of *Rhodobacter sphaeroides*, (submitted)

Laguri, C., Phillips-Jones, M.K. and Williamson, M.P. (2003) Solution structure and DNA binding of the effector domain from the global regulator PrrA (RegA) from *Rhodobacter sphaeroides*: Insights into DNA binding specificity. *Nucl. Acids Res.* **31**, 6778-6787.

Collaborators

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